

Application No.: 10/051,902
Docket No.: BB-1163 US DIV

Page 2

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Amendments to the Specification:

Please amend the following paragraphs:

Title at page 1, line 2:

NUCLEIC ACID ENCODING PLANT SUGAR TRANSPORT PROTEINS

Paragraph at page 2, lines 29-33:

~~Figure 1 shows~~ FIG. 1A, 1B, 1C, 1D and 1E show a comparison of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14 and 16 with the *Arabidopsis thaliana*-like sugar transport protein amino acid sequence set forth in SEQ ID NO:29. Amino acid designations in small case letters represent regions that are thought to be *Arabidopsis thaliana*-like sugar transport protein signatures.

Paragraph at page 2, lines 34-36:

~~Figure 2 shows~~ FIG. 2A, 2B, 2C and 2D show a comparison of the amino acid sequences set forth in SEQ ID NOs:18, 20, 22, 24, 26 and 28 with the *Beta vulgaris*-like sugar transport protein amino acid sequence set forth in SEQ ID NO:30.

Paragraph at page 21, lines 3-7:

~~Figure 1 presents~~ FIG. 1A, 1B, 1C, 1D and 1E present an alignment of the amino acid sequence set forth in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14 and 16 with the *Arabidopsis thaliana*-like sugar transport protein amino acid sequence, SEQ ID NO:29. Alignments were performed using the Clustal algorithm. The percent similarity between the corn, rice, soybean and wheat acid sequences was calculated to range between 16% to 89% using the Clustal algorithm.

Application No.: 10/051,902
Docket No.: BB-1163 US DIV

Page 3

Paragraph at page 22, line 36 though page 23, line 2:

Figure 2 presents FIG. 2A, 2B, 2C and 2D present an alignment of the amino acid sequence set forth in SEQ ID NOs:18, 20, 22, 24, 26 and 28 with the *Beta vulgaris*-like sugar transport protein amino acid sequence, SEQ ID NO:30. Alignments were performed using the Clustal algorithm. The percent similarity between the corn, rice, soybean and wheat acid sequences was calculated to range between 43% to 81% using the Clustal algorithm.

Paragraph at page 23, lines 8-30:

A chimeric gene comprising a cDNA encoding sugar transport protein in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366, date of deposit December 15, 1995. The DNA segment from pML103 contains a 1.05 kb Sall-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-Sall fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding a sugar transport protein, and the 10 kD zein 3' region.

Application No.: 10/051,902
Docket No.: BB-1163 US DIV

Page 4

Paragraph at page 27, lines 19-31:

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol.* 189:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio- β -galactoside, the Inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 μ L of 50 mM Tris-HCl (Tris(hydroxymethyl)aminomethane Hydrochloride) at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One μ g of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

Title at page 30, line 2:

NUCLEIC ACID ENCODING PLANT SUGAR TRANSPORT PROTEINS

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